

OPM-AUTO-1390 (REV 10-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 1702.401600	
INTERNATIONAL APPLICATION NO. PCT/NO99/00141		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/674913 not yet assigned	
INTERNATIONAL FILING DATE April 30, 1999		PRIORITY DATE CLAIMED May 8, 1998	
TITLE OF INVENTION Frameshift Mutants of Beta-Amyloid Precursor Protein and Ubiquitin-B and Their Use			
APPLICANT(S) FOR DO/EO/US Gustav GAUDERNACK, Jon Amund ERIKSEN and Mona MØLLER			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).</p> <p>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p>			
<p>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input checked="" type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> have been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p>			
<p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>			
Items 11 to 16 below concern other document(s) or information included:			
<p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information:</p> <ul style="list-style-type: none"> (i) Sequence Listing - Paper Copy (ii) Sequence Listing - Computer Readable Copy (i.e., diskette) (iii) Statement Under 37 C.F.R. § 1.821(f) (iv) International Search Report (v) International Preliminary Examination Report 			

17. The following fees are submitted:**Basic National Fee (37 CFR 1.492(a)(1)-(5)):**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00

International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 1000 00

Surcharge of **\$130.00** for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

Claims	Number Filed	Number Extra	Rate		
<input checked="" type="checkbox"/> Total Claims	66-20 =	46	X \$18.00	\$ 828	00
<input checked="" type="checkbox"/> Independent Claims	1- 3 =	0	X \$80.00	\$ 0	00
Multiple dependent claim(s) (if applicable)			+ \$270.00	\$ 270	00
TOTAL OF ABOVE CALCULATIONS =				\$	00
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				\$	00
			SUBTOTAL =	\$ 2098	00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 2098	00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$ 2098	00
			Amount to be:		
			refunded	\$	
			charged	\$	

- A check in the amount of \$2098.00 to cover the above fees is enclosed.
- Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1205. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

FITZPATRICK, CELLA HARPER & SCINTO
30 Rockefeller Plaza
New York, New York, 10112-3801
Facsimile: (212) 218-2200



SIGNATURE

John W. Behringer

NAME

23,086

REGISTRATION NUMBER

November 8, 2000

DATE

09/674913

529 Rec'd PCT/PTC 08 NOV 2001

1702.401600

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
GUSTAV GAUDERNACK, ET AL.) : Examiner: Unassigned
Int'l Appln. No.: PCT/N099/00141) : Group Art Unit: Unassigned
Int'l Publn. No.: WO 99/58564) :
U.S. Appln. No.: Unassigned) :
Nat'l Entry Date: November 8, 2000) :
For: FRAMESHIFT MUTANTS OF) November 8, 2000
BETA-AMYLOID PRECURSOR :
PROTEIN AND UBIQUITIN-B)
AND THEIR USE :

Commissioner For Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to the examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 13,

Line 6, change "3" to --2--.

IN THE CLAIMS:

Please delete the originally-filed claims in International Application No. PCT/NO99/00141, as well as Claims 1-26 that were introduced during international prosecution by way of claim amendments made pursuant to Article 19 of the Patent Cooperation Treaty, and which replaced the originally-filed claims, without prejudice to or disclaimer of the subject matter recited in any of these claims.

Please add Claims 27-57 as follows:

--27. A peptide for use in the treatment of Alzheimer's disease or Down's syndrome, said peptide characterized in that it:

a) is a fragment of a mutant β APP protein or a mutant Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease or Down's syndrome, the mutant β APP protein and the mutant Ubi-B protein each having a mutant part and a normal part to its protein sequence;

b) consists of at least one amino acid residue of the mutant part of the mutant β APP protein or the mutant Ubi-B protein;

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c) comprises 0-10 amino acid residues of the mutant β APP protein or the mutant Ubi-B protein corresponding, in the case of either protein, to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and optionally extending to the carboxyl terminus of the mutant part of the protein as determined by a new stop codon generated by the relevant frameshift mutation, wherein the total number of amino acid residues from (b) and (c) is at least 8; and

(d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

28. The peptide according to claim 27, wherein the peptide contains a total of 8-25 amino acid residues.

29. The peptide according to claim 27, wherein the peptide contains a total of 9-20 amino acid residues.

30. The peptide according to claim 27, wherein the peptide contains a total of 9-16 amino acid residues.

31. The peptide according to claim 27, wherein the peptide contains a total of 8-12 amino acid residues.

32. The peptide according to claim 27, wherein the peptide contains a total of 20-25 amino acid residues.

33. The peptide according to claim 27, wherein the peptide contains a total of 9 amino acid residues.

34. The peptide according to claim 27, wherein the peptide contains a total of 12 amino acid residues.

35. The peptide according to claim 27, wherein the peptide contains a total of 13 amino acid residues.

36. The peptide according to claim 27, wherein the peptide is selected from the group of peptides consisting of SEQ ID NO: 1 - SEQ ID NO: 10, and a fragment of any of these peptides.

37. A pharmaceutical composition comprising a peptide according to any of claims 27-36 and a pharmaceutically acceptable carrier or diluent.

38. A vaccine for Alzheimer's disease comprising a peptide according to any of claims 27-36 and a pharmaceutically acceptable carrier or diluent.

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39. The use of a peptide according to claim 27 for the preparation of a pharmaceutical composition for the treatment or prophylaxis of Alzheimer's disease or for the treatment of Down's syndrome.

40. A method for vaccinating a human patient disposed to developing, or afflicted with, Alzheimer's disease, comprising administering to the patient at least one peptide according to claim 27, one or more times, in an amount sufficient to induce specific T-cell immunity to both mutant β APP and mutant Ubi-B proteins or specific T-cell immunity to either of these proteins.

41. A method for vaccinating a human patient disposed to developing, or afflicted with, Alzheimer's disease, comprising administering to the patient at least one peptide according to claims 28-36, one or more times, in an amount sufficient to induce specific T-cell immunity to both mutant β APP and mutant Ubi-B proteins or specific T-cell immunity to either of these proteins.

42. The method according to claim 40, wherein the amount of the at least one peptide is in the range of 1 microgram (1 μ g) to 1 gram (1 g) for each administration.

43. The method according to claim 42, wherein the amount of the at least one peptide is in the range of 1 microgram (1 μ g) to 1 milligram (1 mg) for each administration.

44. A method for treating a human patient afflicted with Alzheimer's disease or Down's syndrome, comprising stimulating the patient *in vivo* or *ex vivo* with at least one peptide according to claim 27.

45. A method for treating a human patient afflicted with Alzheimer's disease or Down's syndrome, comprising stimulating the patient *in vivo* or *ex vivo* with at least one peptide according to any of claims 28-36.

46. The method according to claim 44, wherein the amount of the at least one peptide used is in the range of 1 microgram (1 μ g) to 1 gram (1 g) for each administration.

47. The method according to claim 44, wherein the amount of the at least one peptide used is in the range of 1 microgram (1 μ g) to 1 milligram (1 mg) for each administration.

48. An isolated DNA sequence for use in the treatment of Alzheimer's disease or Down's syndrome comprising a DNA sequence or variants thereof encoding the peptide according to claim 27.

49. An isolated DNA sequence according to claim 48 encoding peptides comprising SEQ. ID. NOS: 1-10 or variants thereof.

50. The use of the DNA sequence according to claim 49, for the preparation of a pharmaceutical composition for the treatment or prophylaxis of Alzheimer's disease or for the treatment of Down's syndrome.

51. A method for treating a human patient disposed to developing, or afflicted with, Alzheimer's disease or afflicted with Down's syndrome, comprising stimulating the patient *in vivo* or *ex vivo* with the DNA sequence according to claims 48 or 49.

52. A vector comprising the DNA sequence of claim 48.

53. The vector according to claim 52, wherein the vector is selected from the group consisting of a plasmid and a viral vector.

54. The vector according to claim 52, wherein the vector is selected from the group consisting of an *E. coli* plasmid, a Listeria vector and a recombinant viral vector.

55. The vector according to claim 54, wherein the recombinant viral vector is selected from the group consisting of an orthopox virus, a canary virus, a capripox virus, a suipox virus, a vaccinia virus, a baculovirus, a human adenovirus, an SV40 virus and a bovine papilloma virus.

56. The use of a plasmid or viral vector according to claim 52 for the preparation of a pharmaceutical composition for the treatment or prophylaxis of Alzheimer's disease or for the treatment of Down's syndrome.

57. A method for treating a human patient disposed to developing, or afflicted with, Alzheimer's disease or afflicted with Down's syndrome, comprising stimulating the patient *in vivo* or *ex vivo* with the vector according to claim 52.--

REMARKS

Applicants request early examination on the merits and favorable consideration of this application.

Claims 27-57 are presently pending in this application, with claim 27 being independent.

The original claims filed in the parent PCT application (i.e., International Application No. PCT/N099/00141 or International Publication No. WO 99/58564) were cancelled during international prosecution without prejudice to or disclaimer of their subject matter, and were replaced with claims 1-26 by way of an amendment made pursuant to Article 19 of the Patent Cooperation Treaty. Claims 1-26 now have also been cancelled without prejudice to or disclaimer of the subject matter recited therein. During international preliminary examination, further claim amendments were proposed pursuant to Article 34 of the Patent Cooperation Treaty. These further amendments can be found in an annex to the International Preliminary Examination Report. A copy of the Report with its annex has been filed herewith. Newly added claims 27-57 introduce the Article 34 claim amendments into the U.S. national phase application. However, the Article 34 claim amendments have been rewritten as claims 27-57 in order to place them in better form under U.S. patent practice. Some of the claims also have been rewritten to reduce the overall number of multiple dependent claims in the application.

Applicants also have made one small amendment in the specification in order to correct an obvious typographical error. Support for the amendment made to the specification can be found,

for example, at page 19, lines 5-10, wherein the proper term, i.e., "Exon 2" is referred to in connection with the genetic sequence in question.

Applicants submit that no new matter has been added by these amendments, and request favorable consideration and early examination of this application on its merits.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 530-1010. All correspondence should be directed to our address listed below.

Respectfully submitted,


John W. Behringer
Reg. No. 23,086
Attorney for Applicants

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30 Rockefeller Plaza
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~~PCT/PCT/PTO 21 JUN 2001~~

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
GUSTAV GAUDERNACK, ET AL.) : Examiner: Alvarado, W.
Int'l Appln. No.: PCT/N099/00141) : Group Art Unit: Unassigned
Int'l Publn. No.: WO 99/58564) :
U.S. Appln. No.: 09/674,913) :
Nat'l Entry Date: November 8, 2000) :
For: FRAMESHIFT MUTANTS OF) June 21, 2001
BETA-AMYLOID PRECURSOR :
PROTEIN AND UBIQUITIN-B)
AND THEIR USE :



BOX PCT
Commissioner For Patents
Washington, D.C. 20231

**SECOND PRELIMINARY AMENDMENT AND RESPONSE
TO EXAMINER'S SUPPLEMENTAL SEQUENCE REQUEST**

Sir:

Applicants respectfully request the Examiner to amend the above-identified application as follows.

IN THE SPECIFICATION:

Delete the Sequence Listing in its entirety [i.e., pages 1-2] and substitute the attached Sequence Listing.

REMARKS

The Sequence Listing filed on November 8, 2000 was not in proper form under U.S. sequence rules set forth in 37 C.F.R. 1.821-1.825. The attached computer readable copy and paper copy of the Sequence Listing, originally submitted on May 25, 2001, and submitted again herewith, are formatted in accordance with U.S. sequence rules.

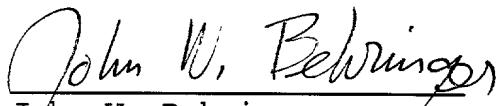
Applicants submit that no new matter is added by the substitute Sequence Listing since the only change is in form.

In a June 15, 2001 voicemail message to the undersigned, Examiner Alvarado stated that he has been unable to find in the file for this application either the diskette copy of the Sequence Listing or the statement under 37 C.F.R. 1.821(f) submitted on May 25, 2001. Accordingly, the following items are enclosed herewith:

- 1) a photocopy of a stamped postcard showing receipt of diskette copy and paper copy of the Sequence Listing and statement under 37 C.F.R. 1.821(f),
- 2) a photocopy of a previously submitted response to the Sequence Listing Notification,
- 3) a photocopy of a previously submitted statement under 37 C.F.R. 1.821(f),
- 4) a supplemental paper copy of the Sequence Listing [as noted above], and
- 5) a supplemental diskette copy of the Sequence Listing [as noted above].

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 530-1010. All correspondence should be directed to our address listed below.

Respectfully submitted,


John W. Behringer
Registration No. 23,086
Attorney for Applicants

FITZPATRICK, CELLA, HARPER & SCINTO
30 Rockefeller Plaza
New York, New York, 10112-3801
Facsimile: (212) 218-2200

Enclosures
JWB/MHW

13 Rec'd PCT/PTO 21 JUN 2001

09/674913

PATENT APPLICATION

1702.401600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
GUSTAV GAUDERNACK, ET AL.) : Examiner: Unassigned
Int'l Appln. No.: PCT/N099/00141) : Group Art Unit: Unassigned
Int'l Publn. No.: WO 99/58564) :
U.S. Appln. No.: 09/674,913) :
Nat'l Entry Date: November 8, 2000) :
For: FRAMESHIFT MUTANTS OF) May 25, 2001
BETA-AMYLOID PRECURSOR :
PROTEIN AND UBIQUITIN-B)
AND THEIR USE :
:

BOX PCT
Commissioner For Patents
Washington, D.C. 20231

STATEMENT UNDER 37 C.F.R § 1.821(f)

Sir:

Applicants hereby submit that the Sequence Listing information recorded in computer readable form on the attached diskette is identical to the paper copy of the Sequence Listing filed concurrently herewith.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 530-1010. All correspondence should be directed to our address listed below.

Respectfully submitted,



Registration No. 30110
Attorney for Applicants

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30 Rockefeller Plaza
New York, New York, 10112-3801
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09/674913

SEQUENCE LISTING

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Gaudernack, Gustav
Eriksen, Jon Amund
Moller, Mona

<120> Frameshift Mutants of Beta-Amyloid Precursor Protein and Ubiquitin-B And Their Use

<130> 001702.401600

<140> US 09/674,913
<141> 2000-11-08

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<151> 1999-04-30

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09/674913
529 Rec'd PCT/PTC 08 NOV 2000

1702.401600

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
GUSTAV GAUDERNACK, ET AL.) : Examiner: Unassigned
Int'l Appln. No.: PCT/N099/00141) : Group Art Unit: Unassigned
Int'l Publn. No.: WO 99/58564) :
U.S. Appln. No.: Unassigned) :
Nat'l Entry Date: November 8, 2000) :
For: FRAMESHIFT MUTANTS OF) November 8, 2000
BETA-AMYLOID PRECURSOR :
PROTEIN AND UBIQUITIN-B)
AND THEIR USE :
:

Commissioner For Patents
Washington, D.C. 20231

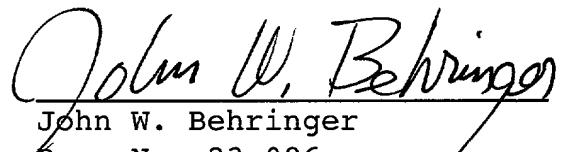
STATEMENT UNDER 37 C.F.R. § 1.821(f)

Sir:

Applicants hereby submit that the sequence listing information recorded in computer readable form on the attached diskette is identical to the written sequence listing filed concurrently herewith.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 530-1010. All correspondence should be directed to our address listed below.

Respectfully submitted,


John W. Behringer
Reg. No. 23,086
Attorney for Applicants

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FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND
UBIQUITIN-B AND THEIR USE

5 The present invention relates to peptides for treatment and/or prophylaxis of Alzheimer's disease and Down syndrome.

Alzheimer's disease and treatment of Down syndrome are both
10 associated with frameshift mutations occurring at the transcriptional level or by posttranscriptional editing of RNA during the encoding of β -Amyloid precursor protein (β APP) and ubiquitin-B (Ubi-B). Such frameshift mutations give rise to mutant β APP and Ubi-B protein products which
15 are characterised by aberrant protein sequences at the carboxyl terminus. Peptides covering, either completely or parts of, the aberrant parts of mutant β APP or Ubi-B protein products elicit T cellular immunity and can therefore be useful in compositions for the treatment of
20 Alzheimer's disease and Down syndrome. Further the peptides of this invention can be used as a prophylactic anti-Alzheimer's disease vaccine.

The invention also relates to DNA sequences encoding
25 peptides corresponding to aberrant β APP and Ubi-B protein sequences found in Alzheimer's disease and Down syndrome patients, and to vectors comprising at least one insertion site containing a DNA sequence encoding at least one such peptide.

30 Further the invention relates to methods for the treatment and/or prophylaxis of Alzheimer's disease by administration of at least one mutant β APP and/or Ubi-B peptide or a recombinant virus vector comprising at least one insertion

site containing a DNA sequence encoding at least one mutant β APP and/or one mutant Ubi-B peptide.

The present invention represents a development of a
5 treatment and/or prophylaxis for Alzheimer's disease based
on the use of peptides to generate activation of the T
cellular arm of the body's own immune system against cells
producing mutant β APP and Ubi-B protein products associated
with Alzheimer's disease.

10

The development and use of the methods for treatment of
Alzheimer's disease may also be directly applicable for
treatment of patients with Down syndrome.

15

Technical Background

Peptides corresponding to aberrant protein sequences
resulting from frameshift mutations in genes in cancer
20 cells elicit specific T cellular immunity and can be used
as anti-cancer vaccines (ref. Norwegian patent applications
filed at the same date as the present application by Norsk
Hydro ASA). In the same manner peptides corresponding to
aberrant protein sequences resulting from frameshift
25 mutations associated with other diseases can be used to
develop treatments of that diseases based on generation of
specific T cellular immunity.

Frameshift mutations result in completely new amino acid
30 sequences in the C-terminal part of the proteins,
prematurely terminating where a novel stop codon appears.
This results in two important consequences:

- 35 1) The truncated protein resulting from the frameshift is
generally nonfunctional, in most cases resulting in
"knocking out" of an important cellular function. Aberrant

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proteins may also gain new functions such as the capacity to aggregate and form plaques. In both cases the frameshift results in disease.

- 5 2) The aberrant new C-terminal amino acid sequence resulting from the frameshift is foreign to the body. It does not exist prior to the mutation, and it only exists in cells having the mutation.
- 10 Since the mutant part of the proteins are completely novel and therefore foreign to the immune system of the carrier, they may be recognized by T-cells in the repertoire of the carrier. So far, nobody has focused on this aspect of frameshift mutations, and no reports exist
- 15 15 on the characterization of frameshift peptides from coding regions of proteins as antigens. This concept is therefore novel and forms the basis for developing vaccines based on these sequences. It follows that such vaccines may also be used prophylactically in persons who inherit defective genes or in other ways are disposed for frameshift mutations. Such vaccines will therefore fill an empty space in the therapeutic armament against inherited forms of disease.
- 20 20
- 25 25 It has been shown that single amino acid substitutions in intracellular "self"-proteins may give rise to tumour rejection antigens, consisting of peptides differing in their amino acid sequence from the normal peptide. The T cells which recognise these peptides in the context of the
- 30 30 major histocompatibility (MHC) molecules on the surface of the tumour cells, are capable of killing the tumour cells and thus rejecting the tumor from the host.

35 In contrast to antibodies produced by the B cells, which typically recognise a free antigen in its native conformation and further potentially recognise almost any

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site exposed on the antigen surface, T cells recognise an antigen only if the antigen is bound and presented by a MHC molecule. Usually this binding will take place only after appropriate antigen processing, which comprises a proteolytic fragmentation of the protein, so that the resulting peptide fragment fits into the groove of the MHC molecule. Thereby T cells are enabled to also recognise peptides derived from intracellular proteins. T cells can thus recognise aberrant peptides derived from anywhere in the cells, in the context of MHC molecules on the surface of the cells, and can subsequently be activated to eliminate the cells harbouring the aberrant proteins.

M. Barinaga, Science, 257, 880-881, 1992 offers a short review of how MHC binds peptides. A more comprehensive explanation of the Technical Background for this Invention may be found in D. Male et al, Advanced Immunology, 1987, J.B. Lippincott Company, Philadelphia. Both references are hereby included in their entirety.

The MHC molecules in humans are normally referred to as HLA (human leukocyte antigen) molecules. They are encoded by the HLA region on the human chromosome No 6.

The HLA molecules appear as two distinct classes depending on which region of the chromosome they are encoded by and which T cell subpopulations they interact with and thereby activate primarily. The class I molecules are encoded by the HLA A, B and C subloci and they primarily activate CD8+ cytotoxic T cells. The HLA class II molecules are encoded by the DR, DP and DQ subloci and primarily activate CD4+ T cells, both helper cells and cytotoxic cells.

Normally every individual has six HLA Class I molecules, usually two from each of the three groups A, B and C. Correspondingly, all individuals have their own selection

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of HLA Class II molecules, again two from each of the three groups DP, DQ and DR. Each of the groups A, B, C and DP, DQ and DR are again divided into several subgroups. In some cases the number of different HLA Class I or II molecules
5 is reduced due to the overlap of two HLA subgroups.

All the gene products are highly polymorphic. Different individuals thus express distinct HLA molecules that differ from those of other individuals. This is the basis for the
10 difficulties in finding HLA matched organ donors in transplantations. The significance of the genetic variation of the HLA molecules in immunobiology is reflected by their role as immune-response genes. Through their peptide binding capacity, the presence or absence of certain HLA molecules governs the capacity of an individual to respond
15 to peptide epitopes. As a consequence, HLA molecules determine resistance or susceptibility to disease.

T cells may control the development and growth of cells producing aberrant proteins by a variety of mechanisms.
20 Cytotoxic T cells, both HLA class I restricted CD8+ and HLA Class II restricted CD4+, may directly kill cells carrying the appropriate antigens. CD4+ helper T cells are needed for cytotoxic CD8+ T cell responses as well as for antibody
25 responses, and for inducing macrophage and LAK cell killing.

A requirement for both HLA class I and II binding is that the peptides must contain a binding motif, which usually is
30 different for different HLA groups and subgroups. A binding motif is characterised by the requirement for amino acids of a certain type, for instance the ones carrying large and hydrophobic or positively charged side groups, in definite positions of the peptide so that a narrow fit with the
35 pockets of the HLA binding groove is achieved. The result of this, taken together with the peptide length restriction

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of 8-10 amino acids within the binding groove, is that it is quite unlikely that a peptide binding to one type of HLA class I molecules will also bind to another type. Thus, for example, it may very well be that the peptide binding motif for the HLA-A1 and HLA-A2 subgroups, which both belong to the class I gender, are as different as the motifs for the HLA-A1 and HLA-B1 molecules.

For the same reasons it is not likely that exactly the same sequence of amino acids will be located in the binding groove of the different class II molecules. In the case of HLA class II molecules the binding sequences of peptides may be longer, and it has been found that they usually contain from 10 to 16 amino acids, some of which, at one or both terminals, are not a part of the binding motif for the HLA groove.

However, an overlap of the different peptide binding motifs of several HLA class I and class II molecules may occur. Peptides that have an overlap in the binding sequences for at least two different HLA molecules are said to contain "nested T cell epitopes". The various epitopes contained in a "nested epitope peptide" may be formed by processing of the peptide by antigen presenting cells and thereafter be presented to T cells bound to different HLA molecules. The individual variety of HLA molecules in humans makes peptides containing nested epitopes more useful as general vaccines than peptides that are only capable of binding to one type of HLA molecule.

Effective vaccination of an individual can only be achieved if at least one type of HLA class I and/or II molecule in the patient can bind a vaccine peptide either in its full length or as processed and trimmed by the patient's own antigen presenting cells.

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The usefulness of a peptide as a general vaccine for the majority of the population increases with the number of different HLA molecules it can bind to, either in its full length or after processing by antigen presenting cells.

5

In order to use peptides derived from an aberrant protein resulting from mutational events in cells as vaccines or therapeutic agents to generate CD4+ and/or CD8+ T cells, it is necessary to investigate the mutant protein in question and identify peptides that are capable, eventually after processing to shorter peptides by the antigen presenting cells, to stimulate T cells.

10

15

Definition of Problem solved by the Invention.

20

25

At present no drug or other treatment is able to eliminate the cells producing the aberrant proteins responsible for the degenerative process in the brain of patients afflicted with Alzheimers disease. There is a continuing need for treatment and prophylaxis of Alzheimer's disease. The present invention will contribute to supply new peptides that can have use as treatment and prophylaxis of Alzheimer's disease. Our approach is aimed at directly eliminating the cells responsible for the degenerative process.

The peptides may also be applicable for treatment of patients with Down syndrome.

30

Definition of the Invention

35

A main object of the invention is to obtain peptides corresponding to peptide fragments of aberrant β APP and

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Ubi-B proteins found in Alzheimer's disease and/or Down syndrome patients which can be used to stimulate T cells.

Another main object of the invention is to develop a treatment for Alzheimer's disease based on the T cell immunity which may be induced in patients by stimulating their T cells either *in vivo* or *ex vivo* with the peptides according to the invention.

A third main object of the invention is to develop a vaccine to prevent the establishment of Alzheimer's disease based solely or partly on peptides corresponding to peptides of the present invention which can be used to generate and activate T cells which produce cytotoxic T cell immunity against cells producing the mutant β APP and mutant Ubi-B proteins.

A fourth main object of the invention is to design a treatment or prophylaxis for Alzheimer's disease specifically adapted to a human individual in need of such treatment or prophylaxis, which comprises administering at least one peptide according to this invention.

A fifth object of the invention is to obtain a treatment for patients with Down syndrome using the same methods as for the treatment of Alzheimer's disease.

These and other objects of the invention are achieved by the attached claims.

Frameshift mutations can occur at the gene level, transcriptional level or by posttranscriptional editing of RNA and result in premature stop codons and therefore a deletion of sometimes large parts of the proteins. Aberrant proteins arising from frameshift mutations have generally

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not been considered to be immunogenic and have therefore not been considered as targets for immunotherapy. Thus it has now surprisingly been found that a group of new peptides corresponding to aberrant proteins resulting from 5 frameshift mutations associated with Alzheimer's disease and Down syndrome are useful for eliciting T cell responses against cells producing such aberrant proteins.

Genes containing a mono nucleoside base repeat sequence, 10 for example of deoxyadenosine bases, or a di-nucleoside base repeat sequence, for example of deoxycytosine-deoxythymidine units, are susceptible to frameshift mutations. The frameshift mutations occur, respectively, either by insertion of one or two of the mono-nucleoside base residue or of one or two of the di-nucleoside base unit in the repeat sequence, or by deletion of one or two of the mono-nucleoside base residue 15 or of one or two of the di-nucleoside base unit from the repeat sequence. A frameshift mutation will from the point of mutation encode a protein with a new and totally different amino acid sequence as compared to the normal protein. This mutant protein with the new amino acid sequence at the carboxy end will be specific for all cells 20 in which such frameshift mutations have occurred.

25 In the remainder of this specification and claims the denomination frameshift mutant peptides will comprise such proteins and peptide fragments thereof.

30 These peptides are at least 8 amino acids long and correspond to frameshift mutant β APP and/or Ubi-B protein sequences associated with Alzheimer's disease and/or Down syndrome.

A peptide according to this invention is characterised in that it

5 a) is at least 8 amino acids long and is a fragment of a mutant β APP and/or Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease or Down syndrome;

and

10 b) consists of at least one amino acid of the mutant part of the mutant β APP and/or Ubi-B protein;

and

15 c) comprises 0-10 amino acids corresponding to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and may further extend to the carboxyl terminus of the mutant part 20 of the protein as determined by a new stop codon generated by the relevant frameshift mutation;

and

25 d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

The peptides of this invention contain preferably 8-25, 30 9-20, 9-16, 8-12 or 20-25 amino acids. They may for instance contain 9, 12, 13, 16 or 21 amino acids.

It is most preferred that the peptides of the present invention are at least 9 amino acids long, for instance

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9-18 amino acids long, but due to the processing possibility of the antigen presenting cells also longer peptides are very suitable for the present invention. Thus the whole mutant amino acid sequence may be used as a

5 frameshift mutant peptide according to the present invention, if it comprises 8 amino acids or more. The invention further relates to a method for vaccination of a person disposed for Alzheimer's disease, consisting of administering at least one peptide of the invention one or
10 more times in an amount sufficient for induction of T-cell immunity to the mutant β APP and/or Ubi-B proteins.

The invention also relates to a method for treatment of a patient with Alzheimer's disease, consisting of administering at least one peptide of the invention one or more times in an amount sufficient for induction of T-cell immunity to the mutant β APP and/or Ubi-B proteins.

15 The invention also relates to a method for treatment of a patient with Down syndrome, consisting of administering at least one peptide of the invention one or more times in an amount sufficient for induction of T-cell immunity to the mutant β APP and/or Ubi-B proteins.

20

Detailed Description of the invention.

In the present description and claims, the amino acids are represented by their one letter abbreviation as known in
30 the art.

The peptides of the present invention are exemplified by the β APP and Ubi-B frameshift mutations associated with Alzheimer's disease and Down syndrome:

In Alzheimer's and Down syndrome patients, intracellular and extracellular deposits of proteins in tangles, neurophil threads and neuritic plaques are correlated with neuronal dysfunction leading to dementia (R.D.Terry et al in *Alzheimer Disease*, R.D.Terry, R.Katzman, K.L.Bick, Eds. (Raven, New York, 1994) pp. 179-196). These protein deposits have been shown to contain forms of β amyloid precursor protein (β APP) and ubiquitin-B (Ubi-B) that are aberrant in the carboxyl terminus, and it has further been shown that these aberrant protein sequences are results of frameshift mutations which probably occur at the transcriptional level or by posttranscriptional editing of RNA (F.W. van Leeuwen et al, Science, vol 279, pp. 242-247).

In the case of β APP two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxyadenosine (GA) unit from the (ACC)GAGAGAGA(ATG) sequence in exon 9, and one by deletion of a GA unit from the (CAT)GAGAGA(ATG) sequence in exon 10.

The mutant β APP peptides resulting from these frameshift mutations are shown in table 1. The peptides with seq id nos 1 and 4 are the mutant part of the β APP protein sequence and the peptides with seq id nos 2, 3 and 5 represent mutant peptides extended into the normal β APP sequence at the amino terminus.

normal β APP; RLEAKHRERMSQVMREWEEAERQAKNLPK
seq id no 1; NVPGHERMGRGRTSSKELA
seq id no 2; RLEAKHRENVPGHERMGRGRTSSKELA
seq id no 3; RLEAKHRENVPGHERMG
seq id no 4; MGRGRTSSKELA
seq id no 5; ERMSQVMRMGRGRTS

Table 1.

Also in the case of Ubi-B two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxythymidine (GT) unit from the (TCT)GAGAGGT(GGT) sequence in exon, and one by deletion of a di-nucleoside deoxycytosine-deoxythymidine (CT) unit from the (TCA)CTCT(GGA) sequence in exon 3. The mutant Ubi-B peptides resulting from these frameshift mutations are shown in table 2. The peptides with seq id nos 6 and 9 are the mutant part of the Ubi-B protein sequence and the peptides with seq id nos 7, 8 and 10 represent mutant peptides extended into the normal Ubi-B sequence at the amino terminus.

normal Ubi-B; HLVLRLRGGMQIFVKTLTGKTITLEVEPSD
seq id no 6; YADLREDPDRQDHHPGSGAQ
seq id no 7; HLVLRLRGYADLREDPDRQDHHPGSGAQ
seq id no 8; HLVLRLRGYADLREDPD
seq id no 9; GGGAQ
seq id no 10; TLTGKTITGGGAQ

Table 2.

The mutant β APP and Ubi-B proteins are only encoded for by cells in which corresponding frameshift mutations have occurred and are therefore targets for specific immunotherapy of Alzheimer's disease and Down syndrome.

According to the present invention, peptides corresponding to mutant β APP and mutant Ubi-B proteins can be used to elicit T cellular immunity and specific killing of cells producing mutant β APP and mutant Ubi-B proteins, which in Alzheimer's disease and Down syndrome patients are correlated with neuronal dysfunction leading to dementia.

Other peptides of the invention can be fragments of the peptides listed in the Tables 1 and 2 above. Such fragments are most preferred from 9-16 amino acids long and include at least one amino acid from the mutant part of the protein.

As used in this description and claims the term fragment is intended to specify a shorter part of a longer peptide or 10 of a protein.

Synthesis

The peptides are synthesised by using continuous flow solid phase peptide synthesis. N-a-Fmoc-amino acids with appropriate side chain protection are used. The Fmoc-amino acids are activated for coupling as pentafluorophenyl esters or by using either TBTU or di-isopropyl carbodi-imide activation prior to coupling. 20% piperidine in DMF is used for selective removal of Fmoc after each coupling. Cleavage from the resin and final removal of side chain protection is performed by 95% TFA containing appropriate scavengers. The peptides are purified and analysed by reversed phase (C18) HPLC. The identity of the peptides is confirmed by using electro-spray mass spectroscopy (Finnigan mat SSQ710).

30

Several other well known methods can be applied by a person skilled in the art to synthesise the peptides.

The peptides of the invention may be used in a method for
35 the treatment of Alzheimer's disease and Down syndrome
patients with cells producing frameshift mutant β APP and
Ubi-B proteins, which treatment comprises administering at

least one peptide of the present invention *in vivo* or *ex vivo* to a patient in need of such treatment.

In another embodiment the peptides of the invention may be
5 used to vaccinate a human being disposed for Alzheimer's disease, by administering at least one peptide of the present invention to said human being.

It is further considered to be an advantage to administer
10 to a human a mixture of the peptides of this invention, whereby each of the peptides of the invention can bind to different types of HLA class I and/or class II molecules of the individual.

15 It is considered that the peptides may be administered together, either simultaneously or separately, with compounds such as cytokines and/or growth factors, i.e. interleukin-2 (IL-2), interleukin-12 (IL-12), granulocyte macrophage colony stimulating factor (GM-CSF), or the like
20 in order to strengthen the immune response as known in the art.

The peptides according to the present invention can be used
25 in a vaccine or a therapeutical composition either alone or in combination with other materials, such as for instance standard adjuvants or in the form of a lipopeptide conjugate which as known in the art can induce high-affinity cytotoxic T lymphocytes, (K. Deres, Nature, Vol.342, (nov.1989)).

30

The peptides according to the present invention may be useful to include in either a peptide or recombinant fragment based vaccine.

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The peptides according to the present invention can be included in pharmaceutical compositions or in vaccines together with usual additives, diluents, stabilisers or the like as known in the art.

5

According to this invention, a pharmaceutical composition or vaccine may include the peptides alone or in combination with at least one pharmaceutically acceptable carrier or diluent.

10

Further a vaccine or therapeutical composition can comprise a selection of peptides which are fragments of the mutant β APP and Ubi-B proteins associated with Alzheimer's disease and Down syndrome.

15

The vaccine according to this invention may further be administered to the population in general for example as a mixture of peptides giving rise to T cell immunity against cells in which Alzheimer's disease and Down syndrome connected β APP and Ubi-B frameshift mutations may occur.

20

The peptides according to this invention may be administered as single peptides or as a mixture of peptides. Alternatively the peptides may be covalently linked with each other to form larger polypeptides or even cyclic polypeptides.

25

A therapy for Alzheimer's disease and Down syndrome according to the present invention may be administered both in vivo or ex vivo having as the main goal to elicit specific T cell immunity against the mutant β APP and Ubi-B gene products associated with Alzheimer's disease and Down syndrome.

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Further, the frameshift mutant peptides of this invention may be administered to a patient by various routes including but not limited to subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous or the like. In one embodiment the peptides of this invention are administered intradermally. The peptides may be administered at single or multiple injection sites to a patient in a therapeutically or prophylactically effective amount.

10

The peptides of this invention may be administered only once or alternatively several times, for instance once a week over a period of 1-2 months with a repeated sequence later, all according to the need of the patient being treated.

The peptides of this invention can be administered in an amount in the range of 1 microgram (1 µg) to 1 gram (1g) to an average human patient or individual to be vaccinated. It is preferred to use a smaller dose in the range of 1 microgram (1 µg) to 1 milligram (1 mg) for each administration.

25 The invention further encompasses DNA sequences which encodes a frameshift mutation peptide.

The peptides according to the invention may be administered to an individual in the form of DNA vaccines. The DNA encoding these peptides may be in the form of cloned plasmid DNA or synthetic oligonucleotide. The DNA may be delivered together with cytokines, such as IL-2, and/or other co-stimulatory molecules. The cytokines and/or co-stimulatory molecules may themselves be delivered in the form of plasmid or oligonucleotide DNA. The response to a DNA vaccine has been shown to be increased by the presence

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of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula :

5 5'-purine-purine-CG-pyrimidine-pyrimidine-3'. Our DNA vaccines may therefore incorporate these or other ISS, in the DNA encoding the peptides, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, *Immunology Today*, 19(2), 89-97).

10

In one embodiment, the DNA sequence encoding the mutant β APP and mutant Ubi-B peptides comprises:

Normal β APP gene sequence (exons 9 and 10).

15

repeat 1

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG

repeat 2

AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA

20

Mutant β APP gene sequence, GA deleted from repeat 1.

GAG AGG CTT GAG GCC AAG CAC CGA GAG AAT GTC CCA GGT CAT GAG
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Mutant β APP gene sequence, GA deleted from repeat 2.

25

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Normal Ubi-B gene (exon) sequence.

deletion motif

30

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACC CTG GAA GTG GAG CCC AGT GAC

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Mutant Ubi-B gene sequence, GT deleted from the deletion motif.

CAC CTG GTC CTG CGT CTG AGA GGG TAT GCA GAT CTT CGT GAA GAC
CCT GAC CGG CAA GAC CAT CAC CCT GGA AGT GGA GCC CAG TGA

5

Normal Ubi-B gene (exon 2) sequence.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
CT repeat
ACC CTG ACC GGC AAG ACC ATC ACT CTG GAG GTG GAG CCC AGT GAC

10

Mutant Ubi-B gene sequence, CT deleted from the CT repeat.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACT GGA GGT GGA GCC CAG TGA

15

The invention further encompasses vectors and plasmids comprising a DNA sequence encoding at least one frameshift mutant β APP and/or Ubi-B peptide. The vectors include, but are not limited to *E.Coli* plasmid, a Listeria vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40, bovine papilloma virus and the like comprising the DNA sequence encoding a mutant β APP and/or Ubi-B peptide.

It is considered that a treatment for Alzheimer's disease and Down syndrome, or prophylaxis for Alzheimer's disease, may be achieved also through the administration of an effective amount of a recombinant virus vector or plasmid comprising at least one insertion site containing a DNA sequence encoding a frameshift mutant peptide to a patient.

whereby the patient's antigen presenting cells are turned into host cells for the vector/plasmid and presentation of HLA/frameshift mutant peptide complex is achieved.

- 5 A person skilled in the art will find other possible use combinations with the peptides of this invention, and these are meant to be encompassed by the present claims.

The peptides according to this invention may be produced by conventional processes as known in the art, such as chemical peptide synthesis, recombinant DNA technology or protease cleavage of a protein or peptide encoded by frameshift mutated β APP gene and Ubi-B gene. One method for chemical synthesis is elucidated in the description below.

Through the present invention the following advantages are achieved:

- 20 It offers a possibility to treat patients suffering from Alzheimer's disease and Down syndrome connected with frameshift mutant β APP and Ubi-B gene products, who known at present do not have any good treatment alternatives.

25 Furthermore it offers a possibility to vaccinate humans prophylactically against the onset of Alzheimer's disease.

SEQUENCE LISTINGCOMMON FOR ALL SEQUENCES.

SEQUENCE TYPE: Peptide

SEQUENCE UNIT: Amino Acid

TOPOLOGY: Linear

SEQUENCE ID NO: 1

SEQUENCE LENGTH: 19 amino acids

N V P G H E R M G R G R T S S K E L A
1 5 10 15**SEQUENCE ID NO: 2**

SEQUENCE LENGTH: 27 amino acids

R L E A K H R E N V P G H E R M G R G R T S S K E L A
1 5 10 15 20 25**SEQUENCE ID NO: 3**

SEQUENCE LENGTH: 17 amino acids

R L E A K H R E N V P G H E R M G
1 5 10 15**SEQUENCE ID NO: 4**

SEQUENCE LENGTH: 12 amino acids

M G R G R T S S K E L A
1 5 10**SEQUENCE ID NO: 5**

SEQUENCE LENGTH: 15 amino acids

E R M S Q V M R M G R G R T S
1 5 10 15

SEQUENCE ID NO: 6

SEQUENCE LENGTH: 20 amino acids

Y A D L R E D P D R Q D H H P G S G A Q
1 5 10 15 20

SEQUENCE ID NO: 7

SEQUENCE LENGTH: 28 amino acids

H L V L R L R G Y A D L R E D P D R Q D H H P G S G A Q
1 5 10 15 20 25

SEQUENCE ID NO: 8

SEQUENCE LENGTH: 17 amino acids

H L V L R L R G Y A D L R E D P D
1 5 10 15

SEQUENCE ID NO: 9

SEQUENCE LENGTH: 5 amino acids

G G G A Q
1 5

SEQUENCE ID NO: 10

SEQUENCE LENGTH: 13 amino acids

T L T G K T I T G G G A Q
1 5 10

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ARTICLE 19 AMENDMENT

AMENDED CLAIMS

[received by the International Bureau on 1st December 1999 (01.12.99);
original claims 1-25 replaced by amended claims 1-26 (5 pages)]

1. A peptide characterised in that it

5 a) is at least 8 amino acids long and is a fragment of a mutant β APP and/or Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease and/or Down syndrome;

10 and

b) consists of at least one amino acid of the mutant part of the mutant β APP and/or Ubi-B protein;

15 and

c) comprises 0-10 amino acids corresponding to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and may further extend to the carboxyl terminus of the mutant part of the protein as determined by a new stop codon generated by the relevant frameshift mutation;

and

25

d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

30

2. A peptide according to claim 1 characterised in that it contain 8-25 amino acids.

3. A peptide according to claim 1 characterised in that it contain 9-20 amino acids.

ARTICLE 19 AND 14

4. A peptide according to claim 1 characterised in that it contain 9-16 amino acids.

5. A peptide according to claim 1 characterised in that it 5 contain 8-12 amino acids.

6. A peptide according to claim 1 characterised in that it contain 20-25 amino acids.

10 7. A peptide according to claim 1 characterised in that it contains 9 amino acids.

8. A peptide according to claim 1 characterised in that it contains 12 amino acids.

15 9. A peptide according to claim 1 characterised in that it contains 13 amino acids.

10. A peptide according to claim 1 characterised in that it 20 is selected from a group of peptides having the following sequence identity numbers:

seq id no. 1 - seq id no. 10 or a fragment of any of these.

25

30

35

ARTICLE 19 Amendment

11. A peptide for use in treatment of Alzheimer's disease or Down's syndrome,

said peptide characterised in that it

5 a) is at least 8 amino acids long and is a fragment of a mutant β APP and/or Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease and/or Down syndrome;

10 and

b) consists of at least one amino acid of the mutant part of the mutant β APP and/or Ubi-B protein;

15 and

c) comprises 0-10 amino acids corresponding to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and may further extend to the carboxyl terminus of the mutant part of the protein as determined by a new stop codon generated by the relevant frameshift mutation;

and

25

d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

30 12. A pharmaceutical composition comprising a peptide according to any of the above claims and a pharmaceutically acceptable carrier or diluent.

13. A vaccine for Alzheimer's disease comprising a peptide according to any of the claims 1-10 and a pharmaceutically acceptable carrier or diluent.

5 14. Use of a peptide according to any of the claims 1-10 for the preparation of a pharmaceutical composition for treatment or prophylaxis of Alzheimer's disease or treatment of Down syndrome.

10 15. Method for vaccination of a person disposed for or afflicted with Alzheimer's disease, consisting of administering at least one peptide according to the claims 1-10, one or more times, in an amount sufficient for induction of specific T-cell immunity to mutant β APP and/or 15 mutant Ubi- β peptides associated with Alzheimer's disease and/or Down syndrome.

20 16. Method according to claim 15 wherein the amount of the peptides is in the range of 1 microgram (1 μ g) to 1 gram (1g) and preferentially in the range of 1 microgram (1 μ g) to 1 milligram (1 mg) for each administration.

25 17. Method for treatment of a patient afflicted with Alzheimer's disease or Down syndrome, by stimulating *in vivo* or *ex vivo* with peptides according to the claims 1-10.

30 18. Method according to claim 17 wherein the amount of the peptides used is in the range of 1 microgram (1 μ g) to 1 gram (1g) and preferentially in the range of 1 microgram (1 μ g) to 1 milligram (1 mg) for each administration.

19. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding a frameshift mutant peptide according to claim 1.

20. An isolated DNA sequence according to claim 19 encoding peptides comprising seq. id. no: 1-10 or variants thereof.

21. Use of a DNA sequence according to any of the claims 5 19-20 for the preparation of a pharmaceutical composition for treatment or prophylaxis of Alzheimer's disease or treatment of Down syndrome.

22. Method for treatment of a person disposed for or 10 afflicted with Alzheimer's disease or afflicted with Down syndrome, by stimulating *in vivo* or *ex vivo* with DNA sequences according to the claims 19-20.

23. A plasmid or virus vector comprising DNA sequences of 15 claim 18 encoding a frameshift mutant β APP peptide and/or Ubi-B peptide associated with Alzheimer's disease or Down syndrome.

24. A vector according to claim 23 wherein the vector is 20 *E.Coli* plasmid, a Listeria vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40 or bovine papilloma virus.

25 25. Use of a plasmid or virus vector according to claim 23 for the preparation of a pharmaceutical composition for treatment or prophylaxis of Alzheimer's disease or treatment of Down syndrome.

30 26. Method for treatment of a person disposed for or afflicted with Alzheimer's disease or afflicted with Down syndrome, by stimulating *in vivo* or *ex vivo* with plasmids or virus vectors according to claim 23.

35



**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

(Page 1)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND UBIQUITIN-B AND THEIR USE

, the specification of which is attached hereto was filed on April 30, 1999 PCT International Application No. PCT/NO99/00141 and was amended on December 1, 1999 (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b), of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designates at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed:

<u>Country</u>	<u>Application No.</u>	<u>Filed (Day/Mo./Yr.)</u>	(Yes/No) <u>Priority Claimed</u>
NORWAY	19982098	8 May 1998	Yes

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

<u>Application No.</u>	<u>Filed (Day/Mo./Yr.)</u>	<u>Status</u> (Patented, Pending, Abandoned)
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I hereby appoint the practitioners associated with the firm and Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to the address associated with that Customer Number:

FITZPATRICK, CELLA, HARPER & SCINTO
Customer Number: 05514

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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